



United States  
Department of  
Agriculture

Animal and  
Plant Health  
Inspection  
Service

Biotechnology  
Regulatory  
Services

4700 River Road  
Riverdale, MD  
20737

Prof. Ian T. Baldwin  
Max Planck Institute for Chemical Ecology  
Department of Molecular Ecology  
Hans-Knöll-Straße 8  
D-07745 Jena  
Germany

Re: Confirmation of the regulatory status of genome edited *Nicotiana attenuata* plants.

Dear Prof. Baldwin,

Thank you for your letter dated September 3, 2018, inquiring whether the *Nicotiana attenuata* (coyote tobacco) product described in your letter is a regulated article under 7 CFR part 340. Your letter describes the *N. attenuata* lines developed through CRISPR/Cas9-mediated genome editing to confer a lack of nectarin (superoxide dismutase) proteins in floral nectar.

The Plant Protection Act (PPA) of 2000 gives USDA the authority to oversee the detection, control, eradication, suppression, prevention, or retardation of the spread of plant pests or noxious weeds to protect the agriculture, environment, and economy of the United States.

USDA regulates the importation, interstate movement and environmental release (field testing) of certain genetically engineered (GE) organisms that are, or have the potential to be, plant pests. Regulations for GE organisms that are or have the potential to be plant pests, under the PPA, are codified at 7 CFR part 340, "Introduction of Organisms and Products Altered or Produced Through Genetic Engineering Which Are Plant Pests or Which There Is Reason To Believe Are Plant Pests." Under the provisions of these regulations, a GE organism is deemed a regulated article if it has been genetically engineered using a donor organism, recipient organism, or vector or vector agent that is listed in §340.2 and meets the definition of a plant pest, or that is an unclassified organism and/or an organism whose classification is unknown, or if the Administrator determines that the GE organism is a plant pest or has reason to believe it is a plant pest.

In your September 3, 2018 letter, you stated that you transformed wild-type *N. attenuata* (Utah accession) with T-DNA containing the gene for the *Streptococcus pyogenes* Cas9 protein and two specific single guide RNAs (sgRNA) targeting three *N. attenuata* genes for the induction of insertion/deletion mutations: *NEC3a*, *NEC1b* and *NEC1d*. You stated that the T-DNA was delivered to your tobacco variety via *Agrobacterium* – mediated transformation and that plant pest sequences were used in the plasmid as regulatory elements. You further stated in your letter that from the parental transgenic plant you obtained two lines: NEC3a-#6, containing two single-base (A) random insertions in the *NEC3a* gene 78 bases apart, and NEC1b1d-4.10, containing a single-base (T) random insertion in the *NEC1b* gene and a single-base (T) random insertion in the *NEC1d* gene. You stated that the two lines were subjected to PCR analysis to confirm that the coyote tobacco plants do not contain the introduced genetic material or any plant pest sequences used during transformation.

Based on the information you provided in your September 3, 2018 letter, USDA concludes that the genome edited *N. attenuata* lines NEC3a#6 and Nec1b1d-4.10 are not plant pests. The

genome edited *N. attenuata* lines do not contain any of the genetic material that was inserted in the GE parent plant for CRISPR/Cas9 editing. The only genetic changes in the genome-edited tobacco lines are two single-base (A) insertions and two single-base (T) insertions. Since no DNA repair template was provided, the resulting insertions were produced by the plant's own naturally-occurring DNA repair mechanism. Therefore, consistent with previous responses to similar letters of inquiry, APHIS does not consider the genome-edited coyote tobacco lines described in your September 3, 2018 letter to be regulated pursuant to 7 CFR part 340.

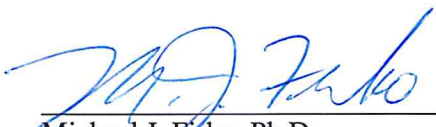
USDA is also authorized to protect American agriculture from damage caused by noxious weeds. If USDA determines that a GE plant or introgression of the GE trait into its sexually compatible wild relatives poses a noxious weed risk, USDA would consider regulating the plant under the noxious weed regulation, 7 CFR part 360. USDA has the option to regulate plants under 7 CFR part 360 regardless of whether or not they meet the definition of a regulated article under 7 CFR part 340. Coyote tobacco is not listed as a Federal noxious weed pursuant to 7 CFR part 360, and APHIS has no reason to believe that the phenotypes resulting from the genome edits described in your letter would increase the weediness of coyote tobacco or its sexually compatible wild relatives.

Please be advised that the importation of the NEC3a#6 and Nec1b1d-4.10 lines of *N. attenuata* seeds or plants, like all other *N. attenuata*, will be subject to Plant Protection and Quarantine (PPQ), permit and/or quarantine requirements. For further information, should you plan to import these lines of *N. attenuata* seeds or plants, you may contact PPQ Permit Services for further information at (877) 770-5990.

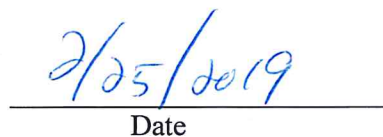
Please be advised that the NEC3a-#6 and Nec1b1d-4.10 lines of *N. attenuata*, while not regulated by APHIS under 7 CFR part 340 may still be subject to other regulatory authorities such as FDA or EPA.

Should you become aware at any time of any issues that may affect the Agency's conclusion regarding this inquiry, you must immediately notify the Agency in writing of the nature of the issue. We hope that you appreciate our commitment to plant health and support for the responsible stewardship for the introduction of GE plants.

Sincerely,



Michael J. Firko, Ph.D.  
APHIS Deputy Administrator  
Biotechnology Regulatory Services  
Animal and Plant Health Inspection Service  
U.S. Department of Agriculture

  
Date